

APPENDIX:

Page 12, paragraph beginning at line 3:

100 mg (0.203 mmol) of epothilon A are dissolved in 3 ml of pyridine, 50 μ l (0.686 mmol) of thionyl chloride are added and the reaction mixture is stirred at room temperature for 15 minutes. 1M Phosphate buffer pH 7 is then added and the aqueous phase is extracted four times with ethyl acetate. The combined organic phases are washed with saturated sodium chloride solution, dried over sodium sulphate and freed of solvent. The crude product is purified and the four stereoisomers **3a-d** are separated by preparative layer chromatography (eluant: toluene/methanol, 90:10).

Page 17, paragraph beginning at line 9:

5 mg (0.009 mmol) of 3,7-di-O-acetyl-epothilon A are dissolved in 1 ml of methanol, 150 μ l of an ammoniacal methanol solution (2 mmol NH_3 /ml methanol) are added and the reaction mixture is

stirred overnight at 50°C.

Page 17, paragraph beginning at line 12:

[To work up] For separation, the solvent is removed *in vacuo*.

The [crude] raw product is purified by preparative layer chromatography (eluant: toluene/methanol, 90:10).

Page 18, paragraph beginning at line 3:

20 mg (0.041 mmol) of epothilon A are dissolved in 0.5 ml of methanol, 0.5 ml of 1N sodium hydroxide solution is added and the reaction mixture is stirred at room temperature for 5 minutes.

Page 18, paragraph beginning at line 6:

The reaction mixture is worked up by adding 1M phosphate buffer pH 7 and extracting the aqueous phase four times with ethyl acetate. The combined organic phases are washed with saturated sodium chloride solution, dried over sodium sulphate and freed of solvent. The [crude] raw product is purified by preparative layer chromatography (eluant : dichloromethane /methanol, 85:15).

Page 19, the paragraph beginning at line 3:

5 mg (0.009 mmol) of 7-O-acetyl-epothilon A are dissolved in 1 ml of methanol, 200 μ l of an ammoniacal methanol solution (2 mmol NH_3 /ml methanol) are added and the reaction mixture is stirred at 50°C for two days. [To work up] For separation, the solvent is removed *in vacuo*. The [crude] raw product is purified by preparative layer chromatography (eluant: toluene/methanol, 90:10).

Page 20, the paragraph beginning at line 4:

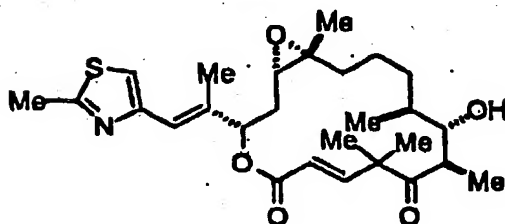
48 mg of epothilonic A acid 7 are dissolved in 6 ml of abs. THF and, with stirring, 40 μ l of triethylamine and 16 μ l of 2,4,6-trichlorobenzoyl chloride are added. After 15 minutes, the precipitate is removed by filtration and the filtrate is added dropwise, within a period of 15 minutes, with rapid stirring, to a boiling solution of 20 mg of 4-dimethylaminopyridine in 200 ml of abs. toluene. After a further 10 minutes, the mixture is concentrated by evaporation *in vacuo* and the residue is partitioned between ethyl acetate/citrate buffer (pH 4). After separation by preparative HPLC, the evaporation residue of the organic phase yields 15 mg of epothilon A.

Page 20, paragraph beginning at line 17:

75 litres of culture are [cultured] grown as described in the basis patent and are used for inoculation [into] in a production fermenter containing 700 litres of production medium consisting of 0.8% starch, 0.2% glucose, 0.2% soya flour, 0.2% yeast extract, 0.1% $\text{CaCl}_2 \times 2\text{H}_2\text{O}$, 0.1% $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, 8mg/litre of Fe-EDTA, pH=7.4 and optionally 15 litres of Amberlite XAD-16 adsorber resin. Fermentation [lasts for from] takes 7 to 10 days at 30°C, with aeration with 2 m³ air/h. The pO₂ is maintained at 30% by regulating the rotary speed.

After page 24:

-- Example 17



[1S-[1R*,3R*(E),10S*,11S*,12R*,16S*]]-11-Hydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadec-6-ene-5,9-dione.

A. [1S-[1R*,3R*(E),7R*,10S*,11S*,12R*,16S*]]-7,11-Hydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione, 7,11-difurmate.

Formic acid (0.95 ml, 25 mmol, 5.0 equiv), 4-N,N-dimethylaminopyridine (1.3 g, 11 mmol), and triethylamine (7.0 ml, 49 mmol) were added to a solution of epothilone B (2.5 g, 5.0 mmol) in CH_2Cl_2 (100 ml). The reaction mixture was cooled to -15°C and acetic anhydride (2.3 ml, 25 mmol) was added over 5 minutes. The reaction mixture was stirred for 15 minutes at -15°C then warmed to room temperature and stirred for 30 minutes. The reaction mixture was quenched with pH 7.0 phosphate buffer, and the organic layer extracted with CH_2Cl_2 (3X100 ml). The combined organic extracts were washed with 1N HCl (1X100 ml) and 10% NaHCO_3 (1X100 ml). The organic layer was dried (Na_2SO_4), filtered, and concentrated under vacuum to give Compound A (2.8 g, 100%), a glassy solid, which was used without further purification.

MS (M+H)⁺ 564

TLC: Rf = 0.71 (9/1 CH_2Cl_2 / acetone, visualization with UV)

B. [1S-[1R*,3R*(E),10S*,11S*,12R*,16S*]]-11-Formyloxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadec-6-ene-5,9-dione, 11-formate.

1,8-Diazabicyclo[5.4.0]undec-7-ene (7.3 ml, 49 mmol, 10 equiv) was added over 5 minutes to a solution of Compound A (2.8 g, 4.9 mmol) in CH_2Cl_2 (140 ml) at room temperature. The reaction mixture was stirred at room temperature for one hour. The reaction mixture was quenched with pH 4.0 phosphate buffer and extracted with CH_2Cl_2 (3X200 ml). The combined organic extracts were washed with 10% NaHCO_3 (1X200 ml). The organic layer was dried (Na_2SO_4), filtered, and concentrated under vacuum to afford Compound B (2.5 g, 100%), a glassy solid, which was used without further purification.

MS (M+H)⁺ 518

TLC: Rf = 0.76 (9/1 CH_2Cl_2 / acetone, visualization with UV)

C. [1S-[1R*,3R*(E),10S*,11S*,12R*,16S*]]-11-Hydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadec-6-ene-5,9-dione.

2M Ammonia in methanol (20 ml, 40 mmol) was added to a solution of Compound B (2.5 g, 4.8 mmol) in methanol (100 ml) at room temperature. The reaction mixture was stirred at room temperature for four hours. The reaction mixture was concentrated under vacuum. The residue was purified by flash chromatography (eluting with 19/1 CH₂Cl₂ / acetone) to afford the title compound (2.1g, 89%), as a glassy white solid.

MS (M+H)⁺ 490

TLC: R_f = 0.41 (9/1 CH₂Cl₂ / acetone, visualization with UV)

Elemental analysis for C₂₇H₃₉NO₅S · 0.22 H₂O

Calc: C, 65.70; H, 8.05; N, 2.84

Found: C, 65.69; H, 8.12; N, 2.77 - -